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Histomorphological evaluation of teat of Sahiwal and Holstein Friesian cattle during lactation and non-lactation stage

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ABSTRACT

The present study was conducted on the teat of Sahiwal and Holstein Friesian (HF) cattle during lactating and non-lactating periods. The samples were collected in 10% NBF, processed to obtain paraffin sections, and stained with various stains. The results revealed that teat skin in Sahiwal and HF cows were reported to be thicker at the teat tip, followed by the mid part and minimum at the base of the teat. The epidermal pegs were pointed and elongated in non-lactating, whereas blunt and flattened in lactating animals. There was stretching of different layers of skin in the lactating animals due to flattened epidermal pegs and dermal papillae. The lining epithelium of teat cistern varied from stratified cuboidal to columnar type and showed mucosal foldings, which were comparatively less in Sahiwal than in HF. The annular folds were well developed in Sahiwal cattle. The lactiferous glands were smaller and lined by low cuboidal epithelium in Sahiwal cows, whereas their number and size were more in the HF. The folds in Furstenberg's rosette varied from 10-14 in Sahiwal and 6-10 in HF cattle. The teat canal epithelium's thickness was higher in HF than in Sahiwal cows. Teat muscle sphincter contained smooth muscle fibres, blood vessels, nerves, and collagen, reticular and elastic fibres. The amount of collagen fibres were relatively more in Sahiwal cattle. It may be concluded that the chances of intramammary infection are comparatively less in Sahiwal cattle as compared to HF ones due to anatomical variations in the formation of annular folds, Furstenberg's rosette and teat sphincter.

Keywords: HF, Histology, Sahiwal cattle, Teat

The basic architecture of the teat skin has been described by Eurell and Frappier (2006). The teat's wall comprises the epidermis, dermis, subcutaneous tissue and mucosa. The epidermis is further composed of stratum basale, stratum spinosum, stratum granulosum and stratum corneum in bovines (Smolenski 2018). The basement membrane separates the dermis from the epidermis and provides tensile strength to the skin. The Fürstenberg's rosette, resembling the rosette of a flower, is present at the junction of the teat canal and teat cistern. The teat canal also called the streak canal, represents a small duct running from the teat sinus to the teat orifice. The teat canal plays a significant role in the defence of the mammary gland against mastitis as it prevents milk leakage and the entry of bacteria (Paul *et al.* 2013). The epithelium of the streak canal was observed to be significantly thick and compact in buffaloes than in cows (Uppal *et al.* 1994). Also, the buffaloes possessed a well-developed smooth muscle sphincter around the streak canal, which helped them in tight closure of the teat canal and thus added to the prevention of mastitis. The outer keratin layer of the streak canal, which formed a keratin plug during the dry period (Smolenski *et al.* 2015), was also measured to be thicker in buffaloes than in cows (Uppal *et al.* 1994). The sub epithelial stroma of the teat canal was composed of collagen fibres with a small amount of elastic and reticular fibres (Naik 2015).

Few studies have evaluated the histomorphological structure of teat in sheep (Modekar *et al.* 2017), goats (Vaish 2012), Malnad Gidda cow (Naik 2015), and buffalo (Uppal *et al.* 1995, Singh 2000). But, to the best of the authors' knowledge, no report has been published with respect to histomorphological observations on the teat skin and teat canal of Sahiwal and HF cattle specific to lactation and non-lactation periods. The present study compares the histomorphological aspects of the teat skin and teat canal in lactating and non-lactating Sahiwal and HF cows.

MATERIALS AND METHODS

Animals and sample collection: The study involved six Sahiwal and six Holstein Friesian (HF) crossbred cows who died due to natural cause or rapid, unexpected death other than mastitis. Based on their history and gross observations, the animals were divided into lactating and non-lactating groups. The fore and rear teat tissue samples were collected from each animal immediately after death and preserved in 10% neutral buffered formalin (NBF) until further processing for histomorphological investigation.

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Histomorphological examination: The histomorphological examination of the teat tissues was carried out using the acetone-benzene technique (Luna 1968). The paraffin sections of 5-6 µm thickness were obtained on clean glass slides and subjected to different histological staining. The stains used were haematoxylin and eosin for routine histomorphological evaluation (Luna 1968), Masson's trichrome for the study of collagen fibres (Luna 1968), and Verhoeff's for elastic fibres, and Gridley's for reticular fibres (Sheehan and Hrapchak 1973).

Microscopy: The microscopic observations were recorded using a camera-mounted Nikon 80i microscope. The required photomicrographs were taken and gauged for micrometry by applying Image J Software. The micrometric evaluation included the teat canal epithelium, teat skin epidermis thickness, and mucosal folds at the level of Furstenberg's rosette. The paired t-test was used to analyze the data (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

Histomorphologically, the teat was made up of teat skin and an internal duct system containing annular folds, a teat cistern, Furstenberg's rosette, and a teat canal as shown in Fig. 1.

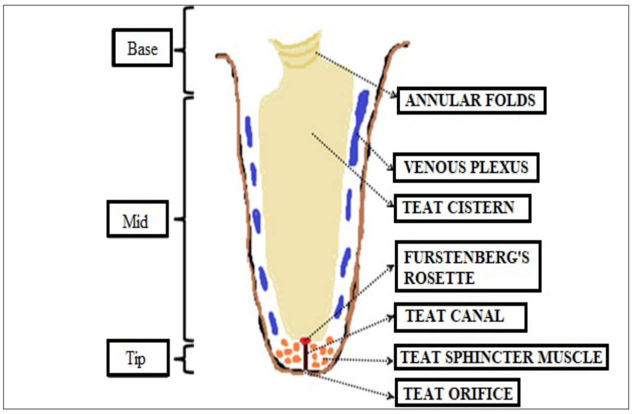


Fig.1. Schematic diagram showing different parts of teat in cattle.

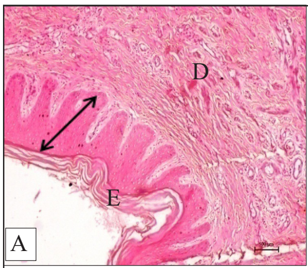
Teat skin: The teat skin comprised the outer epidermis layer and inner dermis (Fig. 2 A). The epidermis of teat skin was stratified squamous keratinized type and made up of stratum corneum, stratum granulosum, stratum spinosum, and stratum basale (Fig. 2 C), whereas the dermis layer contained connective tissue, blood vessels, nerves, muscles, sweat glands (Supplementary Fig. 1 E), sebaceous glands (Supplementary Fig. 1 C), and hair follicles (Fig. 2B). The epidermis was invaginated into the dermis to form the epidermal pegs and dermal papillae (Fig. 2 C). These epidermal pegs were pointed and elongated in non-lactating animals (Fig. 2 C) but blunt and flattened in lactating ones (Fig. 2 D) in both the breeds. These epidermal pegs were further divided into primary and secondary papillae by rete pegs which were more prominent in the teat tip. Similar, the epidermal pegs and dermal papillae were also demonstrated in the skin of buffalo (Debbarma *et al.* 2017) and pigs (Sumena *et al.* 2010) which provide firmness and strength to the teat tip.

The outermost covering of the skin, called the stratum corneum, was strongly eosinophilic and contained flattened, non-nucleated keratinized cells. This layer was thickest at the tip region, followed by the mid part, and minimum at the base of the teat, as shown by arrow in Figs. 2 E, C, A, respectively. The next layer, i.e. stratum granulosum, was made up of 1-2 layers of squamous cells with a flat nucleus and was comparatively thick in the tip region than the mid and base of the teat. These cells contained keratohyalin granules responsible for producing the keratin in the tip and teat canal as explained by Nagaraju *et al*. (2012) in the skin of cattle, goats, and deer. The third layer stratum spinosum was multi-layered, having two to three layers at the base, four at the mid, and six at the tip of the teat. It consisted of polyhedral cells with a spherical nucleus containing melanin granules, which trap and prevent UV rays from entering the skin's dermis, reducing skin malignancies (Singh *et al.* 1975).The last layer stratum basale contained brown staining pigmented cells indicating the presence of melanocytes which were found to be more in the tip region than the base and mid of the teat. Dellmann (1993) explained that the melanocytes in the stratum basale produced melanin pigment, and the amount of pigmentation dropped from the stratum basale to the stratum corneum. The other non-pigmented cell observed in this layer was Langerhans cells with clear cytoplasm and reniform nucleus. These cells were more concentrated near the tip of the teat than at the base and mid regions (Fig. 2 F and Supplementary Fig. 1 A, B). The number and size of Langerhans cells and melanocytes were more in the lactating animals than in the non-lactating. The Langerhans cells were devoid of any pigmented granules and were responsible for the uptake of foreign antigens that cause teat infections and acted as the teat canal's defence mechanism (Romani *et al.* 2012).

There was stretching of different layers of skin in the lactating animals, which formed flattened epidermal pegs and dermal papillae, but no rete pegs were observed at the lactating stage. Similar findings of teat skin's stratified squamous keratinized epithelium were reported in small ruminants (Senthilkumar *et al.* 2020). However, the presence of the stratum lucidum layer demonstrated by these workers in small ruminants could not be seen in cattle in present study.

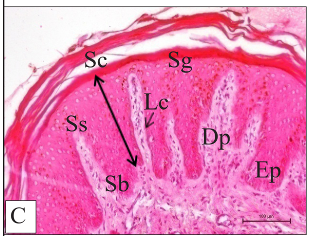
The thickness of teat skin increased significantly ($p<0.01$) from the base towards the tip of the teat in both breeds. Also, the HF, as compared to Sahiwal cows, possessed thicker teat skin ($p<0.01$) at the middle and base parts but not at the tip ($p = 0.05$). The teat skin thickness varied from 252.56±5.51 µm and 279.36±5.46 µm at the base to 257.19±4.92 µm and 304.48±7.65 µm at mid and 369.99±6.56 µm and 355.92±5.75µm at the tip of the teat in Sahiwal and HF cows, respectively.

The skin's dermis contained hair follicles, sebaceous glands and sweat glands in the base region of the teat

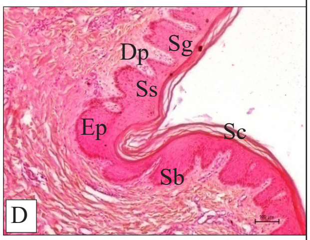


Epidermis (E) and Dermis (D) of base teat skin in Sahiwal

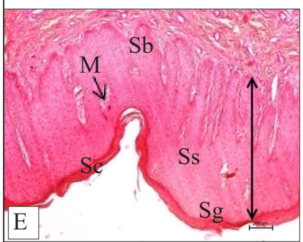
Epidermis (E), Dermis (D), Hair follicle (H) of base teat skin in HF



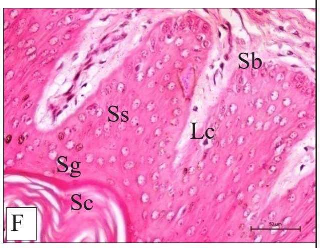
Statum basale (Sb), stratum spinosum (Ss), stratum granulosum (Sg), Stratum corneum (Sc), epidermal peg (Ep), dermal papillae (Dp) of mid skin in non lactating Sahiwal



Epidermal pegs (Ep), Dermal pegs (Dp), Stratum basale (Sb), stratum spinosum (Ss), stratum granulosum (Sg) and Stratum Corneum (Sc) of tip skin in lactating HF



Stratum basale (Sb), stratum spinosum (Ss), stratum granulosum (Sg) and Stratum Corneum (Sc) of tip skin in non lactating HF



Stratum basale (Sb), stratum spinosum (Ss), stratum granulosum (Sg) and Stratum Corneum (Sc) of tip skin in non lactating HF.

Fig. 2. Teat skin (Hematoxylin and Eosin × 100).

skin (Supplementary Fig. 1 C, D, E, F). The hair follicles were simple type and arranged in a linear row in both the breeds and were made up of hair matrix, dermal papillae, and internal and external epithelial root sheath. The dermal connective tissue contained abundant collagen fibres, a few reticular and elastic fibres, blood vessels, smooth muscle, and nerve fibres. The nerve bundles were noticed in the teat dermis at the base, mid, and tip, but their distribution was more at the tip region. In the present study, the dermis of teat skin in the base region was found to contain hair follicles, sebaceous glands, and sweat glands, Similar findings have been reported in the teat skin of sheep and goats (Paramasivan *et al.* 2013), but these structures coulsd not be demonstrated in the teat skin of cattle and buffalo (Uppal *et al.* 1994, Naik 2015, Singh 2000).

Annular folds: These are mucosal folds located at the junction of teat and gland cisterns. Three types of annular folds, viz., large, intermediate, and small were observed in Sahiwal cows (Supplementary Fig. 2 A, B, C). In comparison, HF cows possessed a very thin annular fold lined by simple cuboidal epithelium (Supplementary Fig. 2 D). The larger folds were present primarily in lactating animals and were lined by single-layered columnar epithelium containing lamina propria, muscularis mucosae, tunica submucosa, and tunica muscularis (Supplementary Fig. 2 A). Intermediate folds had a stem with a prominent folding (Supplementary Fig. 2 B). The third type of folds showed the branching pattern and was lined by single-layered cuboidal epithelium (Supplementary Fig. 2 C). Extensive annular folding was observed in Sahiwal cows compared to that in HF cows. Annular folds in bovines were also recorded previously (Singh *et al.* 2020). The annular folds were considered to cause narrowing of the lumen between the teat cistern and gland cistern, thereby inhibiting the milk letdown (Bittner *et al.* 2019). This observation is justified in milking practice with a comparatively slow milk letdown in Sahiwal cows.

Teat cistern: The gland cistern was mainly lined by the single-layered cuboidal epithelium, whereas the teat cistern showed a double-layered cuboidal to columnar epithelium. The teat cistern showed mucosal foldings, which were comparatively more in Sahiwal than in HF cows (Supplementary Fig. 2 E, F). These folds were prominent in non-lactating and were reduced in number and size in lactating ones. This is like Paul *et al.* (2013), who observed numerous longitudinal and circular folds in the mucosa, which became oblique when milk filled in the teat cistern, and these folds were more in local breed cows. The teat cistern was lined by two-layered stratified cuboidal to the columnar epithelium, and sub epithelial stroma contained loose connective tissue of collagen, reticular and elastic fibres along with blood vessels, muscles and nerve bundles (Supplementary Fig. 3 A, B). This is according to Smolenski (2018), who reported a thin double layer of epithelial cells lined the teat cistern epithelium and subepithelial connective tissue.The simple coiled tubular glands termed as the accessory lactiferous glands were present, but their location, size and number differed in the two breeds. These were smaller in size, lined by low cuboidal epithelium, and located at mid and FR regions of the teat cistern in Sahiwal cows. Whereas with locations at the mid, base, and FR regions, their number and size were more in HF. Further, the lactiferous glands were larger in size and contained more eosinophilic secretory materials in lactating than in non-lactating animals (Supplementary Fig. 3 C, D).

Furstenberg's rosette: Furstenberg's rosette was present at the junction of the teat cistern and teat canal. It comprised extensive mucosal folds classified as primary and secondary, projected into the lumen in a rosette form (Supplementary Fig. 3 E). The number of mucosal folds was found more (10-14) in Sahiwal than in HF (6-10). This is similar to the observations of earlier groups (Uppal *et al.* 1994, Paul *et al.* 2013), who described several submucosal rosette-like projections into the teat lumen that varied from 10-14 in number and were more in local breed cows than in crossbred cows. A higher number of folds in Jenubi cows (10-12) than in crossbred cows (7-9 folds) was also reported (Alsodany *et al.* 2019). Vesterinen *et al.* (2015) demonstrated that cows with larger teat sinus cavities had wider rosettes and more mucosal folds. Van der Merwe (1985) explained that Fürstenberg's rosette was a valve to retain milk in the teat. The FR was lined by double-layered columnar epithelium and its stroma contained abundant collagen fibres with few reticular and elastic fibres and different cellular components such as polymorphonuclear cells, lymphocytes, monocytes, plasma cells, and mast cells. The aggregations of lymphoreticular tissues were also seen at the tip of FR in both breeds (Supplementary Fig. 3 F). These aggregations of lymphoreticular tissues present in FR played an essential role in producing antibodies that act as a barrier between the bacteria and epithelium (Nickerson and Pankey 1983).

Teat canal: The lining epithelium of the teat canal was stratified squamous keratinized type and was made up of single layer stratum basale, 2-4 layers of stratum spinosum, and 5-7 layers of stratum granulosum (Supplementary Fig. 4 A). All these layers of the teat canal epithelium were covered by keratinized layer, which was more prominent in non-lactating animals. Teat canal epithelium also contained a few vesicular components known as Marksaulchen cells. Their number and size decreased towards the lumen of the teat canal, and these were found more in lactating cows (Supplementary Fig. 4 C) than in non-lactating. This follows Fürstenberg (1868), who first observed the presence of circular vesicle-like structures emanating from the supra-papillary plate between the rete ridges throughout the teat canal epithelium. Our findings are further strengthened by the observations of Mańkowski (1903), who labeled these cells as Marksäulchen and demonstrated them as distinguishing features of the teat canal epithelium. These structures are thought to provide enhanced flexibility to the teat canal epithelium (Forbes 1968). A higher amount of keratohyalin granules was also noticed in the stratum granulosum of the HF teat canal epithelium than in Sahiwal cattle. The epithelium of teat canal was made up of a single layer of stratum basale, 2-4 layers of stratum spinosum, and 5-7 layers of stratum granulosum. It is like Mańkowski (1903), who also observed a single layer of stratum basal cells, and the stratum spinosum was made up of many layers of polyhedral-shaped cells. Chandler *et al.* (1969) found the stratum granulosum in the teat canal at least five times thicker than in the teat.

The finger-like projections from the teat canal epithelium into the subepithelial tissue were called rete ridges. The latter was long and pointed in non-lactating animals, whereas blunt and wider in lactating animals (Supplementary Fig. 4 A, B). The purpose of rete ridges in the epidermis was to enhance the surface area of contact between the epithelium and the dermis, allowing efficient nutrition transfer to epithelial cells (Elgharably *et al.* 2013). Further, these structures provided mechanical stability and resistance to the shearing pressures for the epithelial surface (van Zuijlen *et al.* 2002) and acted as protective niches for the keratinocyte stem cells found at the base of the major rete ridges (Xiong *et al.* 2013).

The lumen of the teat canal was star-shaped in non-lactating animals and round to oval-shaped in lactating cattle. The teat canal was surrounded by circular smooth muscle bundles recognized as teat sphincter (Supplementary Fig 4 E). In some places, the longitudinal smooth muscle bundles were also observed between the circular muscles. The teat sphincter also contained connective tissue, blood vessels and nerves. The amount of collagen fibres increased in Sahiwal cattle than in HF (Supplementary Fig. 4 F). The reticular and elastic fibres were seen in the basement membrane of the stratum basale and the subepithelial connective tissue. The amount of reticular fibres was higher in lactating animals than in non-lactating ones. The presence of teat sphincter around the teat canal had also been recorded in cattle and buffaloes (Uppal *et al.* 1995). The teat sphincter maintains the tight closure of the teat canal in-between milkings to prevent leakage and keep keratin occluding the teat canal lumen, thus preventing organisms from progressing upward into the teat cistern (Akers and Nickerson 2011). Teat canal closure is a dynamic process governed by a spiraling network of smooth muscle and elastic fibres that encircles the teat canal and lower teat sinus (Van der Merwe 1985). Further, we observed more keratin material in non-lactating than in lactating cows. This could be explained by Capuco *et al.* (1990) that as the milk production increased in cows, stronger shearing forces during milking were expected to remove more keratin from the teat canal, resulting in reduced keratin in lactating animals. In cows where the keratin plug failed to develop, the teat canal remained open and increased the risk of intramammary infections (Williamson *et al.* 1995). The presence of nerve bundles in the teat sphincter resulted in hormonal-nerve stimulation during the suckling and milking of the animal (Ferdowsi *et al.* 2017).

The average thickness of the teat canal epithelium (403.39±3.76 vs 364.99±4.78 µm) and luminal diameter (1804.34±11.11 vs 1274.48±25.37 µm) differed significantly ($p<0.01$) between HF vs Sahiwal cows.

It may be concluded from the present study that the teat skin was thickest at the teat tip, which decresed in mid part and became minimum at the base of the teat. Furstenberg's rosette consisted of primary and secondary folds, which were more (10-14) in Sahiwal than in HF cattle (6-10). The teat canal epithelium was found thicker in HF than in Sahiwal cows. The teat muscle sphincter was very well-organized in both breeds and contained many smooth muscle fibres, blood vessels and nerves. The amount of collagen fibres found were more in the teat sphincter of Sahiwal than that of HF cattle. It may be inferred that the chances of intramammary infection are less in Sahiwal breed than HF due to anatomical differences in number of annular folds, Furstenberg's rosette and composition of teat sphincter.

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